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Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats

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There is ample evidence from basic research and animal carcinogenicity studies that heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) are mutagens and carcinogens. However, there was a paucity of human data due to a lack of appropriate investigative tools. We developed the first validated cooked meat module within a food frequency questionnaire (FFQ) in the United States of America and created databases to be used in conjunction with this FFQ to estimate intake of HCAs and benzo[*a*]pyrene, a marker of PAHs. It became clear that other aspects of meat may also contribute to carcinogenesis; in particular, we are pursuing two additional areas: processed meat and iron exposure in relation to cancer risk. To investigate these hypotheses, we have expanded the cooked meat module to include detailed information on processed meats and fish. In addition, we are developing two databases, one for total iron and heme iron in cooked meat and the other for nitrite, nitrate, and *N*-nitroso compounds in processed meats. In this report, we will outline the methods used to develop the meat questionnaires, the databases, a software package for generating the intake values, and the methods used to generate nutritional data from nationally representative samples.

Keywords: Cooking methods / Food frequency questionnaire / Heterocyclic aromatic amines / Meat / Preserved meat / Polycyclic aromatic hydrocarbons / Preserved meat

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1 Introduction

Red meat has generated a great deal of scientific interest for several years with respect to its association with a variety of cancers. In the past decade, there have been several reviews on meat consumption as a risk factor for several cancers, including two large consensus reports from the World Cancer Research Fund [1] and the Committee on Medical Aspects of Food and Nutrition Policy [2], although both panels agreed that the epidemiology was not consistent. In an effort to clarify the proposed associations between meat consumption and cancer risk, and to identify the underlying mechanisms of these relationships, we are developing methods to improve dietary intake estimates of possible carcinogens.

There are several plausible biological mechanisms to explain an association between meat consumption and cancer [3]. This association may be due to a combination of factors such as the content of fat, protein, iron, and/or meat preparation (*e.g.*, cooking or preserving methods). Laboratory results have shown that meats cooked at high temperatures contain potential mutagens in the form of heterocyclic amines (HCAs) [4–8] and polycyclic aromatic hydrocarbons (PAHs) [9]. To investigate the role of these compounds, we have created separate databases for HCAs and PAHs, which we have used in conjunction with a validated

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Abbreviations: B[*a*]P, benzo[*a*]pyrene; **CSFII**, continuing survey of food intake by individuals; **DiMeIQx**, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; **FFQ**, food frequency questionnaire; **HCA**, heterocyclic amines; **HHHQ**, health habits and history questionnaire; **MeIQx**, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; **MMQ**, meat module questionnaire; **NDL**, nutrient data laboratory; **NHANES**, national health and nutrition examination survey; **NOC**, *N*-nitroso compound; **PAH**, polycyclic aromatic hydrocarbon; **PhIP**, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; **USDA**, United States Department of Agriculture

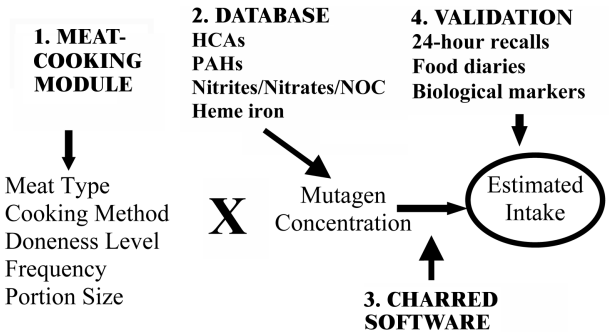

Figure 1. Method used to estimate intake of mutagens.

meat-cooking food frequency questionnaire (FFQ). Red meat may also be associated with cancer by contributing to *N*-nitroso compound (NOC) exposure [3]. Humans can be exposed to NOCs by exogenous routes (from processed meats in particular) and by endogenous routes. Endogenous exposure to NOCs is dose-dependently related to the amount of red meat in the diet. Furthermore, meat in the diet makes a substantial contribution to iron exposure, which may be a cancer risk factor [10].

In order to investigate the various meat-related hypotheses, we needed to develop tools (Fig. 1) to investigate meat cooking and meat-related mutagens (*e.g.*, HCAs and PAHs), as well as investigating the role of processed meat. In this manuscript, we will outline how we developed a meat module within a FFQ. We will also describe how the databases for these compounds were formed and used with the software we developed (CHARRED) to estimate the intake of the different meat-related compounds. We will also provide information on how to obtain nationally representative sampling schemes for creating databases.

2 FFQ meat module development

We used two approaches in developing the meat module questionnaire (MMQ). For meat-cooking mutagens, we modified existing FFQs, while for processed and preserved meats we used survey data to define the relevant food items pertaining to the questions in the FFQ.

2.1 Meat cooking, HCAs, and PAHs

The two most important elements in HCA production are cooking temperature and time [8, 11]. We found that the cooking technique could serve as a reasonable proxy of temperature and doneness level as a surrogate for time. The main determinant of PAH formation in cooked meat is the cooking technique. Early epidemiologic studies found a suggestive association between meat cooking techniques and cancer risk but the information obtained on meat cooking practices could not differentiate factors that had an important influence on the production of HCAs and PAHs. For example, roast beef and steak were included within the same meat category, despite widely dissimilar cooking methods and very different levels of HCA formation. The MMQ consists of questions regarding the consumption of 23 meat/poultry and fish items using a matrix format and includes questions on the frequency of consumption, portion size, and cooking methods (Table 1a). The meat cooking method information indicated whether the meat was pan-fried, grilled/barbecued (by placing it on a grid over coals, open fire, or ceramic briquettes heated by gas), oven-broiled (by placing it below the heat source) baked/roasted, or microwaved. For those red meats that are typically cooked in a standard way, for example, beef stew, meat loaf, liverwurst, and luncheon meat, no cooking method information was collected. Certain meats are commonly cooked to various degrees of doneness level including hamburger/cheeseburger, beefsteak, pork-chops/ham-steaks, sausages, hot dogs, and bacon; therefore, we embedded questions within the questionnaire to determine how well these meats were usually cooked. A set of photographs of different meats and doneness levels was developed as a visual aid [4, 5] to use in conjunction with a matrix in the questionnaire (Table 1b). A group of males and females aged 60 – 75 years were shown these photographs and asked to rank them in categories of rare, medium-rare, medium, medium-well, well, and very well-done. We then chose four photographs indicating both the internal coloring and external browning for hamburgers and steak, and three photographs of external browning for pork-chops/ham-steaks, sausage/hot dogs, bacon, and chicken. This questionnaire allows us to estimate the intake of specific types of meat, HCAs, PAHs, and mutagenic activity.

2.2 Processed meats

Using the continuing survey of food intake by individuals (CSFII 1994 – 96), 104 smoked and processed meat food codes were identified that represented meats consumed in the United States from 1994 to 1996. After collapsing the food codes that linked to the same ingredient food (*e.g.*, three types of bologna), 94 smoked and processed meat items remained. Market checks were conducted to identify a representative food product for each of the 94 meat items. Food products of various brands were found for 82 of the 94 meat items; for each of the meat items, the nutrition label was used to identify additives present in the meat item. The additives identified included: benzoic acid; sodium diacetate; butylated hydroxyanisole; mono- and disodium phosphate; butylated hydroxytoluene; sodium erythorbate and erythorbic acid; citric acid; sodium lactate; lactic acid starter culture; sodium nitrate; potassium tripolyphosphate;

Table 1a. An example of food frequency questionnaire for steak

Type of meat and cooking method	How often										How much			
	Never	Less than once per month	1 per Mo.	2–3 per Mo.	1 per Wk.	2 per Wk.	3–4 per Wk.	5–6 per Wk.	1 per Day	2+ per Day	A medium serving equals	Your serving size		
												S	M	L
Beef steaks Pan-fried: Grilled/barbecued: Oven-broiled: Other (specify): Don't know											4 oz.			

Table 1b. Steaks cooked by different methods and doneness levels to link with the FFQ to develop database for HCAs and PAHs

	Rare	Medium	Well-done	Very well-done
Pan-fry				
Oven-broil				
Grill/barbecue				

sodium nitrite; mono- and dipotassium phosphate; sodium tripolyphosphate; sodium ascorbate and ascorbic acid; sorbic acid, sodium sorbate, and calcium sorbate.

The meat items were then reviewed for frequency of consumption and grouped on the basis of the type of meat and food additive composition. This resulted in 17 meat categories: bacon, sausage, bologna, hot dogs, smoked fish, deli ham, turkey ham, other turkey or chicken cold cuts, salami, pepperoni, beef luncheon meats, other cold cuts, corned beef, baked ham, liverwurst, smoked turkey, and meat spread/potted meat. Nine of these categories were then subdivided according to the differences in the additives present in the meats. For example, the bologna meat category was split into five types: low-fat bologna, beef bologna, pork bologna, turkey bologna (which was then grouped with "other turkey or chicken cold cuts"), and all other types of bologna based on their additive composition. A total of 17 main questions and 22 subquestions were developed representing each of the meat categories and the types of meat in specific categories. The processed meats questions were added to the meat questionnaire which already included questions about frequency of consumption, cooking methods, and degree of doneness for hamburgers, cheeseburgers, pork chops, beef steak, bacon, sausage, and chicken.

2.3 Cognitive testing

The MMQ was then tested by nine subjects for understandability and usability. This cognitive testing involved the subjects talking through their thought patterns as they were filling out the questionnaire. The testing revealed the need to change food terminology and rephrase questions for understandability. The revised questionnaire refined the chicken questions and added additional meat, fish, gravy, and mixed dish categories in four sections: breakfast, lunch, dinner, and mixed dishes and casseroles. The refined questionnaire and accompanying meat doneness photo card were tested in a second round of cognitive interviews by eight subjects, which resulted in some final formatting and text changes to the questionnaire and meats card.

3 Developing databases

When we first began working in this field, there was no database available to examine the content and possible mechanisms of action of cooked and preserved meat by-products. To address this issue, we have developed databases that could be used in conjunction with the FFQ to estimate intake of meat-related mutagens. These are well-developed databases available for meats consumed in the United States of America and are being used by a number of epidemiologic studies.

3.1 HCA database

For each meat question in the questionnaire module, we had multiple samples cooked by different methods to varying degrees of doneness by nutritionists at the Human Nutrition Research Center, U.S. Department of Agriculture (USDA), Beltsville, MD, USA. Because the amount of mutagen formed varies from one piece of meat to another, multiple samples were cooked at different sessions for each cooking method and doneness level. Approximately 2500 individual pieces of meat were cooked to provide data for 120 categories by cooking method and doneness that were ultimately used to create the HCA database. The levels of various HCAs (*e.g.* 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]-

quinoxaline (DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were measured in duplicate by solid-phase extraction and analyzed by high-performance liquid chromatography by the method of Gross and Gruter [12, 13], described in detail by Knize *et al.* [14]. HCA recoveries for each sample were determined from the average of duplicate samples spiked with all five compounds. The identities of peaks at the retention time of known HCAs were confirmed by photodiode-array ultraviolet spectra in all cases. The investigators measuring HCA content were blinded to the type of meat, cooking method, and degree of doneness.

Rather than taking the total sum of HCAs as the measure of total HCA exposure, we used total measured mutagenic activity in the composite meat samples by the Ames *Salmonella* assay. Total mutagenic activity gives greater weight to highly mutagenic compounds. The details of HCA content in the various types of meat samples are contained in our published reports [4, 5, 14 – 17]. In summary, we found that: (i) 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) were not detectable in any of the meat samples; (ii) the measured values of the specific HCAs varied with meat type, cut of meat, cooking method, and doneness level; (iii) the different HCAs were formed in varying amounts, with PhIP being the most abundant and DiMeIQx the least; (iv) HCAs generally increased with doneness; (v) very high levels of PhIP are formed in chicken cooked to very well-done by methods, such as grilling/barbecuing.

3.2 PAHs/benzo[*a*]pyrene (B[*a*]P) database

PAHs are ubiquitous in our diet and therefore, in addition to meat items, other foods were measured for B[*a*]P. Further details are described in Kazerouni *et al*. [9]. In summary, each food item in the FFQ was a composite sample with the proportions derived from the second national health and nutrition examination survey (NHANES II). The store-bought non-meat items included vegetables, fruits, cereals, grains, breads, sweets, dairy and fat products that were fresh, frozen, or cooked.

The composite samples were saponified in alcoholic potassium hydroxide, extracted into isooctane, cleaned up by liquid-liquid partitioning and column chromatography, and analyzed *via* thin-layer chromatography/fluorescence, as described previously [9, 18, 19], to detect the B[*a*]P content. To verify that B[*a*]P is a good marker for other PAHs in food items, we calculated the correlation coefficients between B[*a*]P values of a representative sample of food items and their carcinogenic PAH values or total PAH values. Selected samples were analyzed for the total PAHs, naphthalene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene and cyclopenta[*cd*]pyrene in our study food samples. The correlation coefficient between the total PAH values and the B[*a*]P value was 0.87 ($P = 0.0001$). To determine the correlation between B[*a*]P and other carcinogenic PAHs, we measured the concentrations of benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, chrysene, and cyclopenta[*cd*]-pyrene in the study food samples. The correlation coefficient between the carcinogenic PAH values and the B[*a*]P value was 0.98 ($P = 0.0001$). The highest levels of B[*a*]P (up to about 4 ng B[*a*]P/g of cooked meat) were found in well-done grilled meat The B[*a*]P level in non-meat items was generally low. However, certain cereals and greens, for example kale and collard greens, had levels up to 0.5 ng/g [9], which is substantial when considering the average portion size.

3.3 Total iron and heme iron database

We have completed the first stage of the development of a total iron and heme iron database for meat items. Our motivation to create this database was due to published meta-analyses, and animal carcinogenicity studies that showed an increased risk of various tumors with red meat consumption but no association with white meat intake. The major difference between red and white meat is a considerably higher amount of iron in red as compared to white meat. Dietary iron exists in two main forms: non-heme iron and heme iron. Non-heme iron is found mainly in cereals, vegetables, and meat, whereas heme iron is found primarily in meat as part of hemoglobin and myoglobin. Heme iron can be partially converted to non-heme iron depending on heat treatment and the type and the extent of cooking method [20 – 22]. The meat samples that were cooked for the creation of the HCA and PAH databases provided an exceptional opportunity to estimate heme iron content in relation to cooking. We measured total iron and heme iron by atomic absorption spectrometry for these samples. In preliminary analyses, we found that chicken had the lowest level of total and heme iron and steak and hamburger had the highest levels, with the pork values in between the two.

3.4 Nitrate, nitrite, and NOC database

We are in the process of developing a database for nitrate, nitrite, and NOCs, which are all compounds found in processed meats. Curing meats has been used for centuries as a method of preservation and generally involves adding salt, nitrate, or nitrite to the food. Nitrite is added to processed meat as an antibacterial agent against *Clostridium botuli-*

num and as a cosmetic agent to react with myoglobin to produce the characteristic red-pink color of cured meats. Nitrate and nitrite added to meat can form NOCs, which are known carcinogens [23].

In order to develop the database for the processed meat questions in the MMQ, ten meat types were identified that constituted 90% of the total number of mentions of processed meats in the CSFII 1994 – 1996. "Mentions" is defined as the number of times a food item was reported in a 24-h dietary recall collected in CSFII. A total of 23 USDA survey food codes were then selected from within these ten meat types that represented 90% of all "mentions" for each meat type. The USDA nutrient data laboratory (NDL) provided the corresponding National Nutrient Databank numbers for each survey food code. For each meat type, a composite was prepared using brand name meats that were available from grocery stores. Where major brand name products were not available, a local/regional or store brand product was substituted.

4 Analyses software: computerized heterocyclic amines resource for research in epidemiology of disease (CHARRED)

A software application, CHARRED (www.charred.cancer.gov), was developed to compute the HCA and PAH values from meat intake. The application also supplies information for each of the compounds allowing the investigator to examine which meat items are contributing the most to each compound. The meat database in CHARRED contains each meat type, portion size, HCA/PAH values, and a designator for red or white meat. CHARRED allows the user to add additional meats to the database that they wish to inspect and also has the ability to add additional compounds. Hot dogs and fish were not included in the database; hot dogs produced minimal amounts of HCAs and PAHs and fish resulted in inconsistent data because of the skin sticking to the surface of the pan during cooking.

Each of the meat items in the database are broken down into cooking method and doneness level and their corresponding HCA and PAH values. The doneness levels were grouped into four categories for hamburger and steak: rare, medium, well-done, and very well-done. Medium rare was assigned rare and medium-well was assigned to medium. Chicken, pork chops, bacon, and sausage were grouped into three levels of doneness: just, well, very well. Fried chicken and gravy were not assigned doneness levels. The HCAs and PAHs in the database are: MeIQx, PhIP, DiMeIQx, actual mutagenicity, predicted mutagenicity, and B[*a*]P. Chicken was more comprehensive since the list was differentiated by the factors defined in the questionnaire, including chicken with bones and without bones, chicken with skin and without skin, white meat and dark meat. These chicken samples were categorized by three methods: grilled/barbecued, broiled, pan-fried. Each of these methods was entered into the meat database by three doneness levels and by three skin categories: with skin, without skin, and both (mean of skin and no skin).

Two portion files were initially developed. The first portion file used the meat portions in the Block-NCI health habits and history questionnaire (HHHQ) [24, 25] database; therefore, the portions for all the methods of each meat type were the same. The second method considered the moisture losses during cooking that we had recorded. After reviewing the analysis from both portion size files, it was concluded that there was no significant difference. Therefore, the portion sizes based on the Block-NCI HHHQ database was used for future studies. In addition, portion sizes are available by age and gender, which are also based on the Block-NCI HHHQ database.

4.1 Analyzing data using the meat questionnaire and database

The meat consumption frequency is used for the analysis and the individual frequencies for each cooking method were weighted appropriately to sum up to the main meat frequency given. In addition, several questionnaires allowed the respondent to write-in 'other' cooking method practices not listed on the questionnaire. These methods were converted to those that best matched a line item in the meat database.

Imputations were made for method of cooking as well as doneness level for respondents who did not answer a method of preparation or doneness level for a question they did give an intake frequency for. The imputations for both cooking method and doneness level were dependent upon the most common method and doneness for specific meat items reported for the particular study because eating practices vary between different regions of the country. A gram percentage was used for any mixed dishes containing meat, such as spaghetti and beef stew, since they contain other non-meat products.

5 Validation of the MMQ

We conducted several methodologic projects to assess the validity of the meat cooking questions for estimating HCA intake and to identify areas in the module for improvement. We designed a validation study for the MMQ for HCAs and selected nutrients in 165 healthy participants in a study conducted at the National Navy Medical Center [26]. We used 12-day food diaries (three sets of four nonconsecutive day

diaries completed over a 3-month period) as the reference method. Crude correlation coefficients of HCA intake assessed by the FFQ and food diaries ranged from 0.22 to 0.43, and the de-attenuated correlations ranged from 0.36 to 0.60. We found that although the meat module underestimated absolute MeIQx and PhIP intake, its ability to rank individuals according to intake was reasonable. For example, classification of individuals by the FFQ and multiple diaries into the same or adjacent quintile was 70% for MeIQx and 63% for PhIP intake. In contrast, misclassification into extreme quintile occurred in less than 6% of the group for MeIQx and PhIP intake.

Using the MMQ, we have investigated the role of meat, HCA, and PAH intake in cancer development in several studies. We found that HCAs were significantly associated with tumors of the colorectum, breast, stomach, pancreas, and lung and that B[*a*]P was associated with an increased risk of colorectal adenoma [27 – 34].

6 Nationally representative databases

Much of the data generated for HCAs and PAHs has been limited to meats bought locally and cooked by nutritionists at the USDA. Local sampling does have its limitations as there may be differences in meat composition, which may affect the formation of HCAs or PAHs. However, the majority of the variation in HCA or PAH level in meat is more likely to be dependent on the cooking method and doneness level, any regional variations in these would be captured by our questionnaire. In addition, the processed meat database was generally formed from brand name products, which will be the same throughout the country. Nevertheless, we do need to expand our effort to a nationally representative food composition database. Suitable models for database development can be adapted from the efforts of the USDA, Agricultural Research Services, which develops and maintains the National nutrient databases. USDA has been actively collecting and/or generating, evaluating, and compiling representative food composition data for more than 100 components of foods. The NDL, Beltsville Human Nutrition Research Center, provides USDA's National nutrient database for standard reference (www.nal.usda.gov/fnic/foodcomp), the foundation of most other food composition databases, including the Food and Nutrient Dietary Data System for the NHANES [35]. In addition, efforts have resulted in several special interest databases which provide the composition of foods for a single class of components (*e.g.* flavonoids) [36]. These databases are an essential resource to investigate the potential health effects of various components and can be used as a model for the development of databases for levels of suspected harmful compounds in foods. The process of developing the databases for new food components requires the acquisition of well-documented, accurate, and representative data [37]. Documentation should include a detailed and specific food description, details of sampling, number of samples collected, and sample handling (*e.g.*, processing and preparation steps). Data to be used to assess the intake of dietary components on a nationwide basis should be generated from nationally representative samples [38]. Furthermore, documentation is needed about the chemical analysis of food samples to include a description of sample handling procedures, analytical methods, and analytical quality control procedures. Objective documentation of data sources and methods can then be evaluated to ascertain data quality, an important dimension of the development of accurate and precise databases for health research.

Since 1997 the USDA has conducted the National Food and Nutrient Analysis Program to generate new original data for frequently consumed foods which are major sources of important nutrients in the U.S. diet [38, 39]. Foods are sampled in 12 cities in supermarkets, restaurants, and even homes of participants in selected studies to obtain sample units which represent the national food and beverage supply. As an example, raw ground beef products were sampled from the supermarkets nationwide to generate new nutrient values for this product. Ground beef is a major contributor of protein, iron, vitamins B6 and B12, as well as choline and selenium [40, 41]. Ground beef is the major form of beef consumed in the U.S., accounting for more than 50% of all beef consumed. Ground beef is prepared and sold in major fast-food restaurant chains as well as in the home. Since 1990 ground beef sold in the supermarket has changed, reflecting the preference for leaner meat products. Today, ground beef products in the marketplace range in the level of fat from 5 to 30% of total weight. To sample ground retail beef the nationally based-sampling plan covered four regions of the U.S., with three strata per region [38]. Within each strata a generalized consolidated metropolitan statistical area (gCMSA) was identified and two locations within each of these was selected. These gCMSAs were derived from the U.S Census, 1990. Ground beef products were purchased nationwide and sent to the University of Wisconsin where they were logged into a data system, and prepared for cooking, where applicable, and processed for analysis. Products were cooked by four methods, namely broiled patties, pan-broiled patties, pan-browned crumbles, and baked loaves. Cooking temperatures and degrees of doneness were limited to those recommended by the National Cattlemen's Beef Board, a trade association of the beef industry.

Nutrient analyses on raw and cooked product were performed on samples classified by each fat category. Regression equations were developed to estimate nutrient content of ground beef products at any fat level. The new nationally representative ground beef data were released in Standard

Reference 15 (2002) of the National nutrient database for standard reference and are carried forward in successive releases of the Standard Reference [35, 42]. These data are being used as part of the USDA's Food and Nutrition Dietary Data System to calculate the intake of nutrients from foods containing ground beef which have been reported by participants.

Databases for other types of dietary components can be generated by sampling foods from retail supermarkets and commercial establishments in proportion to their consumption by the U.S. population. Data generated by the chemical analysis of component levels, using valid and accurate methods can provide the basis for calculations to estimate intake of components in a free-living population.

7 Conclusions

In the last decade we have developed questionnaires and databases for specific compounds found in meat. This work has led to advancement in our understanding of cancer epidemiology in relation to meat-related carcinogens. These approaches can be used as a model to develop specific questionnaires and databases for other compounds in the diet that are not normally assessed, for example, phytochemicals in the diet.

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